vaccine delivery at any dose. Two patients vaccinated with the lowest vaccine dose showed stable disease during more than a year. The patients with the intermediate and highest vaccine doses are still monitored with respect to clinical response. The results of CTL and T helper response assays against the individual vaccine components will be presented.

1302

Immune responses to peptide-based cancer vaccines

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Three classes of antigens recognized by cytotoxic T lymphocytes (CTL) are defined in melanoma and some other tumors: 1. Cancer-testis (CT) antigens (MAGE, BAGE, GAGE), expressed in tumors and testis, 2. Differentiation antigens (Melan A/MART-1, tyrosinase gp100/Pmel17, gp75), expressed in melanoma and melanocytes 3. Antigens defined by mutations (CDK-2/R24C, MUM-1, β-catenin). Target structures for CTL recognition are peptides of 9–10 aminoacids length. These peptides bind to MHC class I molecules and are presented at the tumor cell surface. Synthetic peptides can generate specific CTL in vitro that effectively lyse tumor cells expressing the corresponding antigen. Clinical trials using MAGE-1 and MAGE-3 peptides for immunization in HLA-A1+ patients with MAGE-expressing tumors showed objective responses (CR/PR) in some melanoma patients. We immunized HLA-A2+ melanoma patients with peptides derived from Melan A/MART-1, tyrosinase, and gp100/Pmel17. Delayed-type-hypersensitivity reactions (DTH) and peptide-specific CTL responses as well as objective tumor regressions were observed in 3/12 patients. Subsequently, the effects of systemic GM-CSF on immune reactions to peptide vaccines were assessed. Enhanced DTH reactions were observed with infiltrates of CD4+ and CD8+ T lymphocytes, CD1a+ Langerhans cells, and a strong expression of IL-2 and γIFN, suggesting the activation of CD4+ Th1 and CD8+ CTL. Objective tumor regressions were documented in 5/16 patients. The identification of further tumor associated antigens recognized by CTL and the use of adjuvants to enhance their immunogenicity will open broad perspectives for the development of polyvalent cancer vaccines to control or inhibit tumor growth in vivo and to prevent tumor escape of antigen-loss variants.

1303

Active immunization of melanoma patients with IL-2- OR IL-4-transduced allogeneic melanoma cells

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The aim of these clinical studies was to immunize stage IV melanoma patients with HLA-A2-compatible, immunogeneic human melanoma lines genetically modified to release IL-2 or IL-4 in order to elicit or increase a T cell-mediated anti-melanoma response which may affect distant melanoma lesions. These lines were characterized for transgene expression and for the presence of immunological relevant molecules before in vivo vaccination. Twelve patients were treated with IL-2 releasing line while 10 patients were treated with IL-4 gene transduced melanoma cells. The side effects of the treatment were locally mild and systemically absent. All patients were assessable for clinical response and received at least 3 vaccine administrations. Three and 2 mixed responses were clinically observed in group of patients treated with IL-2 and IL-4 transduced melanoma lines, respectively. To evaluate specific immune response, limiting dilution experiments and mixed tumor-lymphocytes cultures were performed using different HLA-A2 compatible melanoma lines, autologous line when available and peptides obtained from known melanoma antigens. This immunological monitoring was performed on peripheral blood lymphocytes obtained from each patient before and after vaccination. An increased frequency of lytic and specific lymphocyte precursors was observed in some cases. Histological and immunohistochemical analyses of biopsies obtained before and after vaccination from tumor nodules and sites of vaccine injection are in progress and will be presented.

1304

Mutant ras peptide vaccines

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ras proto-oncogenes activated by point mutations within codon 12, 13 or 61 are frequently found in human tumours. Since mutant p21 ras molecules encoded by these oncogenes are specific for cancer cells, mutant p21 ras or ras-derived peptides are attractive candidates for a cancer vaccine.

We have studied T cell responses to mutant p21 ras in both healthy volunteers and in cancer patients for the purpose of developing therapeutic and prophylactic cancer vaccines. From these studies we were able to define optimal peptides for T cell stimulation that contain overlapping epitopes capable of stimulating both CD4 and CD8 T cell responses. Studies with a large number of T cell clones derived from different donors, including cancer patients demonstrated that binding of ras derived peptides to HLA class II molecules is promiscuous. Together these results indicated that immunotherapy targeted against neoplastic cells carrying ras mutations is possible.

We have now completed a pilot phase I/II clinical study, and in some of the patients we were able to elicit an immune response by vaccination with autologous ras-peptide loaded antigen presenting cells. The responding cells were both of the CD4 Th1 and CD8 phenotype and were able to kill autologous tumor cells as well as other tumor cells carrying the same ras mutation (12Gly→Val) These results indicate that ras peptide vaccination may result in the generation of a potentially beneficial immune response in cancer patients. We are presently conducting several new clinical protocols based on ras peptide vaccination of patients with different forms of cancer, either by direct intradermal injection of peptides and using recombinant human GM-CSF as adjuvant, or by intralymphatic injection of peptide pulsed dendritic cells.

1305

Oncogene activation as a prognostic marker in head and neck cancer

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In cancer numerous chromosomal abnormalities (e.g. deletions, amplifications and translocations) are associated with the (in-)activation of tumorsuppressor and oncogenes. The detection of genetic aberrations has clinical relevance in classifying tumors or as prognostic factor. For instance, carcinomas of the head/neck region (HNSCC) frequently show amplification of EGFR and the chromosome 11q13 region, point-mutations in p53 and loss of $p16^{INK4}$. We have focused on DNA amplification of the chromosome 11q13 region that was found in 36% of HNSCC. (1) Comparison of HNSCC-patients with and without DNA amplification revealed that amplification is correlated with poor prognosis. (2) We have identified two genes *cyclin D1* and *EMS1/cortactin* that are overexpressed due to DNA amplification. For a proper understanding of the biological behavior of tumors with 11q13 amplification, we introduced these genes into cells *in vitro* to study the effect on (cell) biological properties. (3) With antibodies against the gene products, we have developed an immunohistochemical screening method that is evaluated by comparisons to southern blot and interphase FISH data. Reliable and easier detection methods will enable us to screen large series of HNSCC, to refine the classification of relevant tumors, and ultimately to design more rational therapies. All these aspects will be discussed.

1306

Predicting response in head and neck cancer: The search for the Holy Grail

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A standard radiation therapy schedule is not the optimal treatment for all patients presenting with head and neck (H&N) cancer. To modify the treatment parameters, predictive tests are required allowing to discriminate subpopulations of patients for whom the modification of treatment parameters could be beneficial. We intend to review the current status on predictive tests especially aimed at determining proliferation status (PS),